Saskatchewan
Agriculture
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AGRICULTURE DEVELOPMENT FUND

FINAL REPORT

19990110

ACCELERATING THE DEVELOPMENT OF GLUCOSINOLATE FREE BRASSICA NAPUS

Funded by: The Agriculture Development Fund

March 2007

Prepared by: Agriculture and Agri-Food Canada

Accelerating the Development of Glucosinolate free Brassica napus

Principal Investigator
Dr. Derek Lydiate
Agriculture and Agri-Food Canada
107 Science Place
Saskatoon, S7N 0X2

Project Period
October 1999 to January 2003.

(5659) of 224 individuals that were each self pollinated to produce F3 families of seed (Figure 1). The glucosinolate content of F3 seed was analyzed and the seed glucosinolate character was shown to be segregating in the 5659 population. Objective 2: The AAFC Saskatoon Research Centre, with funding from the Matching Investment Initiative and the Canola Breeding Industry pioneered the large scale development of microsatellite markers for Brassica crops. These markers have been demonstrated to be highly informative and highly reproducible and they have been widely adopted by the Canola Breeding industry. The specificity of microsatellite markers is controlled by the precise DNA sequences represented in the oligonucleotide primer pairs for each marker. The project adopted microsatellites as the marker of choice and a total of 1530 primer pairs where screened to identify those that were informative (that is amplified polymorphic loci) in the 5659 population. 572 informative primer pairs were identified. The marker genotypes of 94 selected F2 plants were established using the majority of the informative primer pairs. This analysis identified 268 robustly scoreable loci and led to the development of a genetic map from the 5659 population. When the genetic marker data was correlated with the data on seed glucosinolate content generated from the F3 seed it was possible to identify three strong QTL's contributing to the genetic control of ULSG. These QTL's were on chromosomes (linkage groups) N7, N10 and N12 of the B. napus genome. Objective 3: The genetic analysis described above identified robust microsatellite markers closely linked to each of the three loci controlling ULGS: sN3620 was associated with the ULGS gene on N7, sORH13 was associated with the ULGS gene on N10 and sN1982 and sC200 flanked the ULGS gene on N12. Although it would be highly desirable to develop specialized populations to map each of the three QTL's to a higher resolution the four microsatellite markers identified above (and associated markers flanking each of them) can already be used to select for inheritance of the genes controlling the ULGS character in breeding programs with the target of developing advanced Canola.

(b) Executive summary

The objective of the project as stated in the original proposal was as follows:

"The objective of the project is to develop marker-assisted breeding approaches in *B. napus* to accelerate the introgression of genes/alleles controlling the zero total glucosinolates trait into high yielding, high quality lines. The glucosinolate free meal will enhance the value of Canadian canola and make it more competitive on domestic and international markets. As well, this project will create biotechnology tools that will accelerate delivery of this novel, high quality trait to the Canadian canola industry."

The project has achieved both of the research goals contained within this objective. The first by developing marker-assisted breeding approaches to accelerate the introgression of genes/alleles controlling the ultra-low seed glucosinolate trait through the genetic mapping of the genes controlling this trait. The second by identifying biotechnology tools in the form of microsatellite markers that will accelerate delivery of the ultra-low seed glucosinolate trait to the canola industry. For details please see the Abstract above and the Technical Report below.

N7; sORH13 associated with the ULGS gene on N10; and sN1982 and sC200 that flank the ULGS gene on N12.

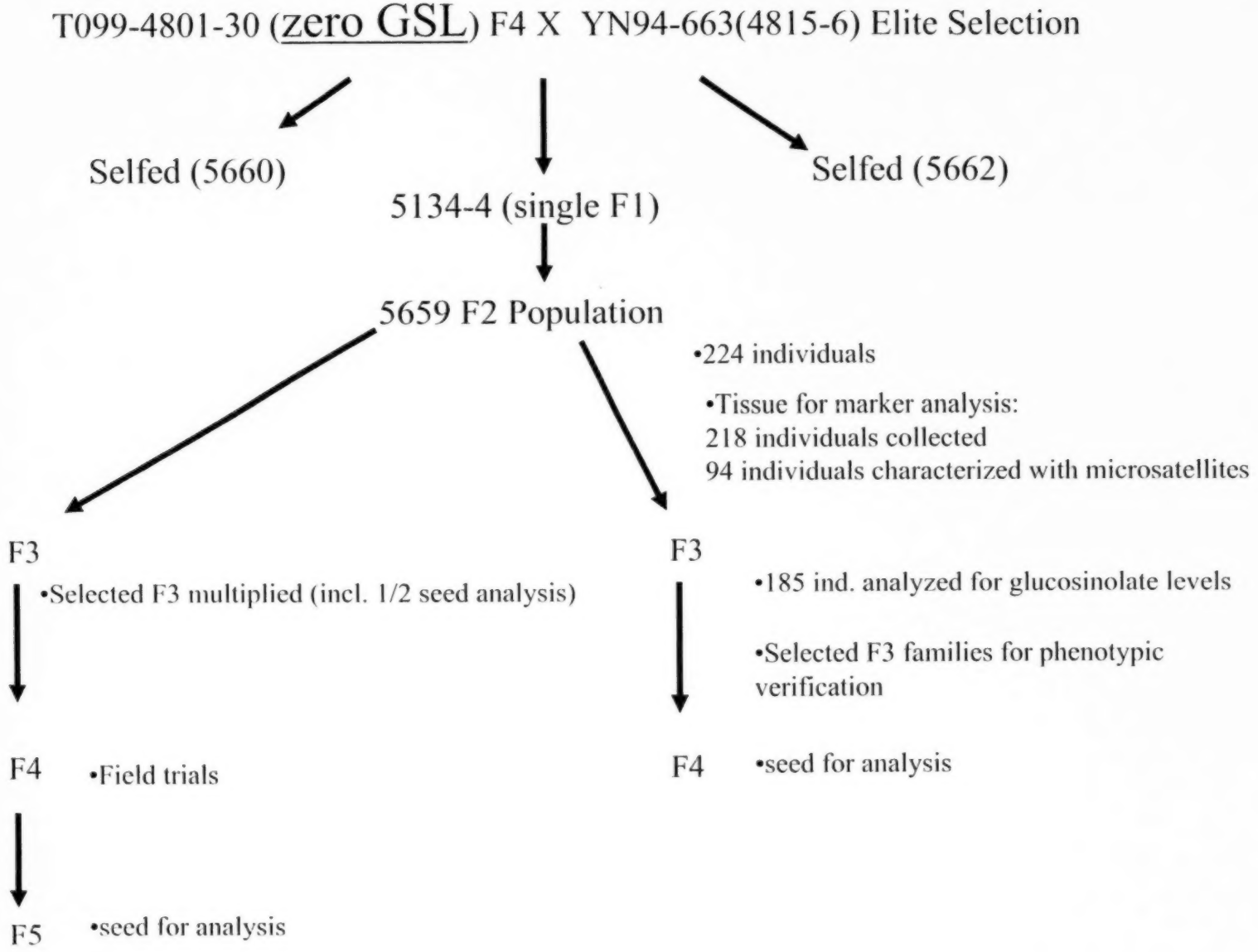


Figure 1: Pedigree of plant lines used in the genetic analysis of the ultra-low seed glucosinolate content character in *B. napus*

Figure 2: Frequency Distribution of Tot. GSL

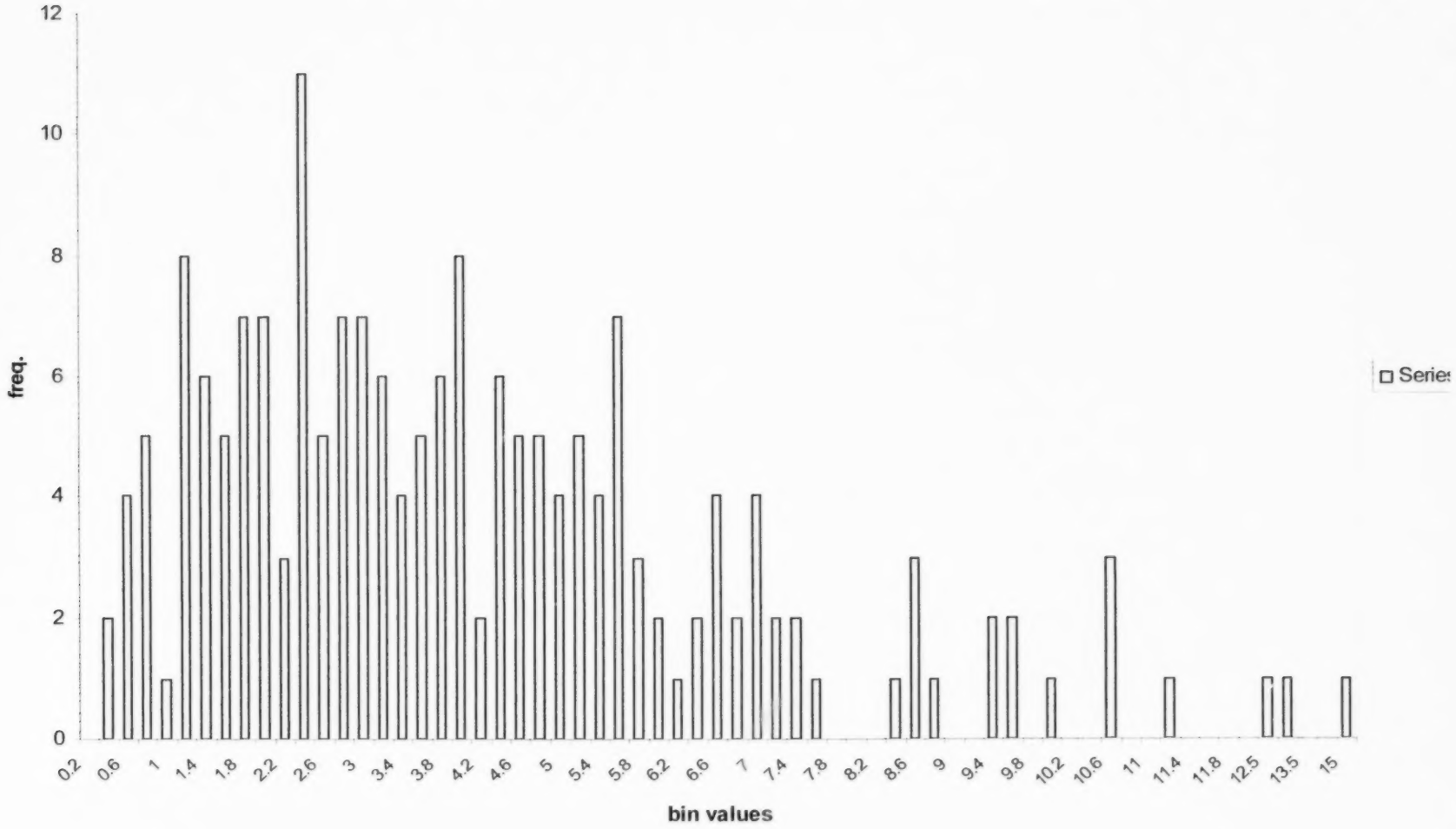
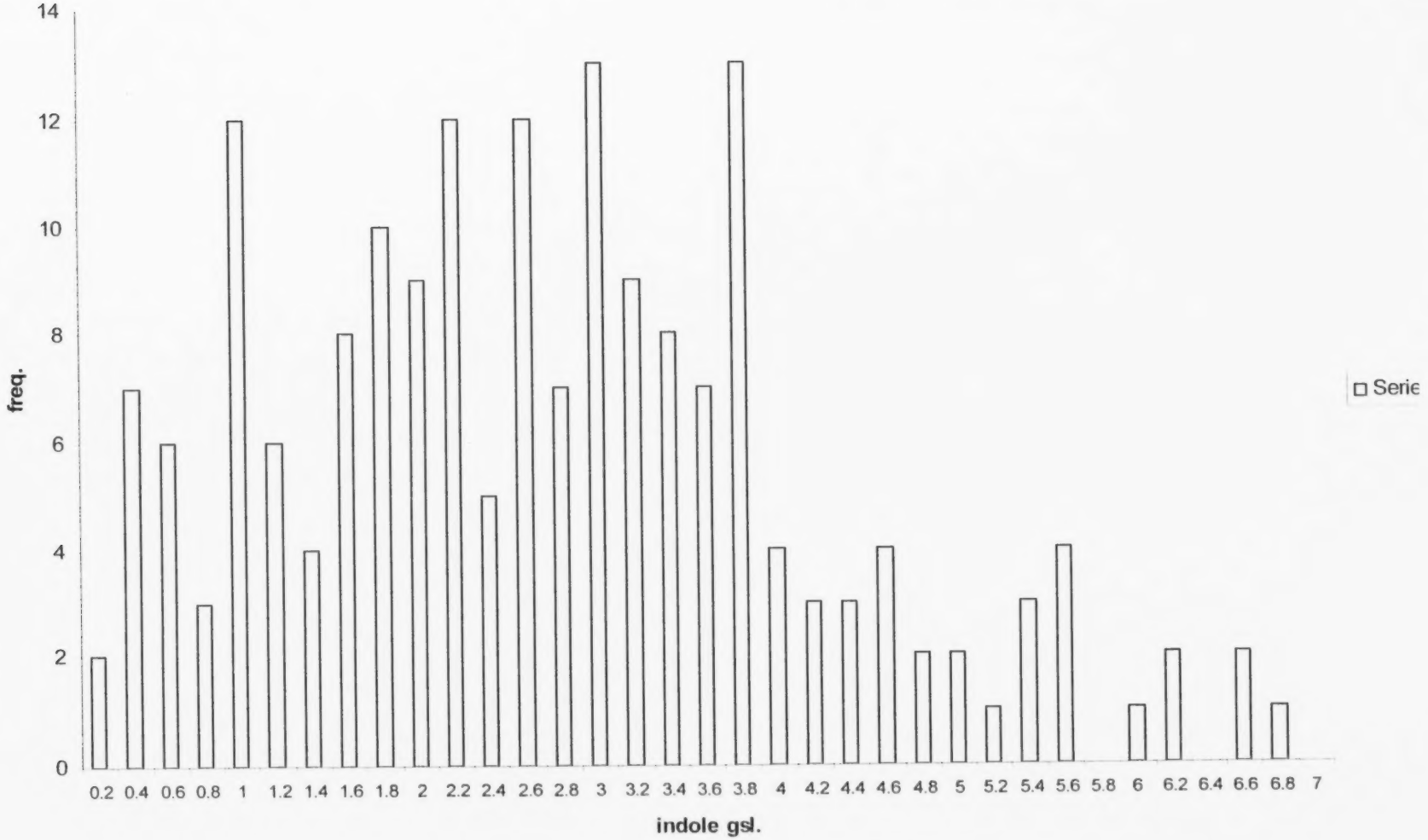


Figure 3: Frequency distribution of Indole gluc.



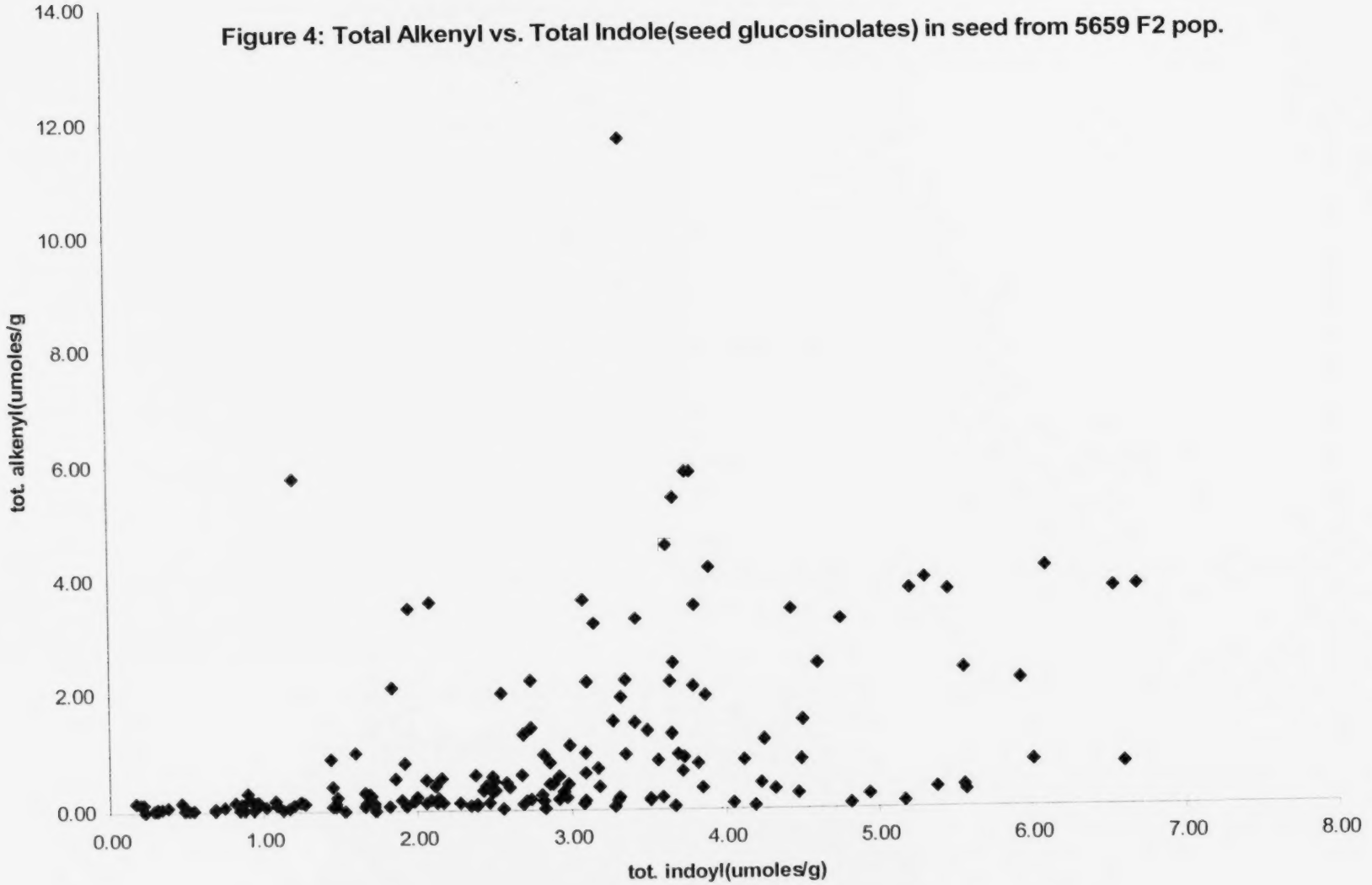


Figure 4: Total Alkenyl vs. Total Indole(seed glucosinolates) in seed from 5659 F2 pop.
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
tot. alkenyl(umoles/g
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
tot. indoyl(umoles/g)

Figure 5: Segregation ratios for loci with co-dominant alleles

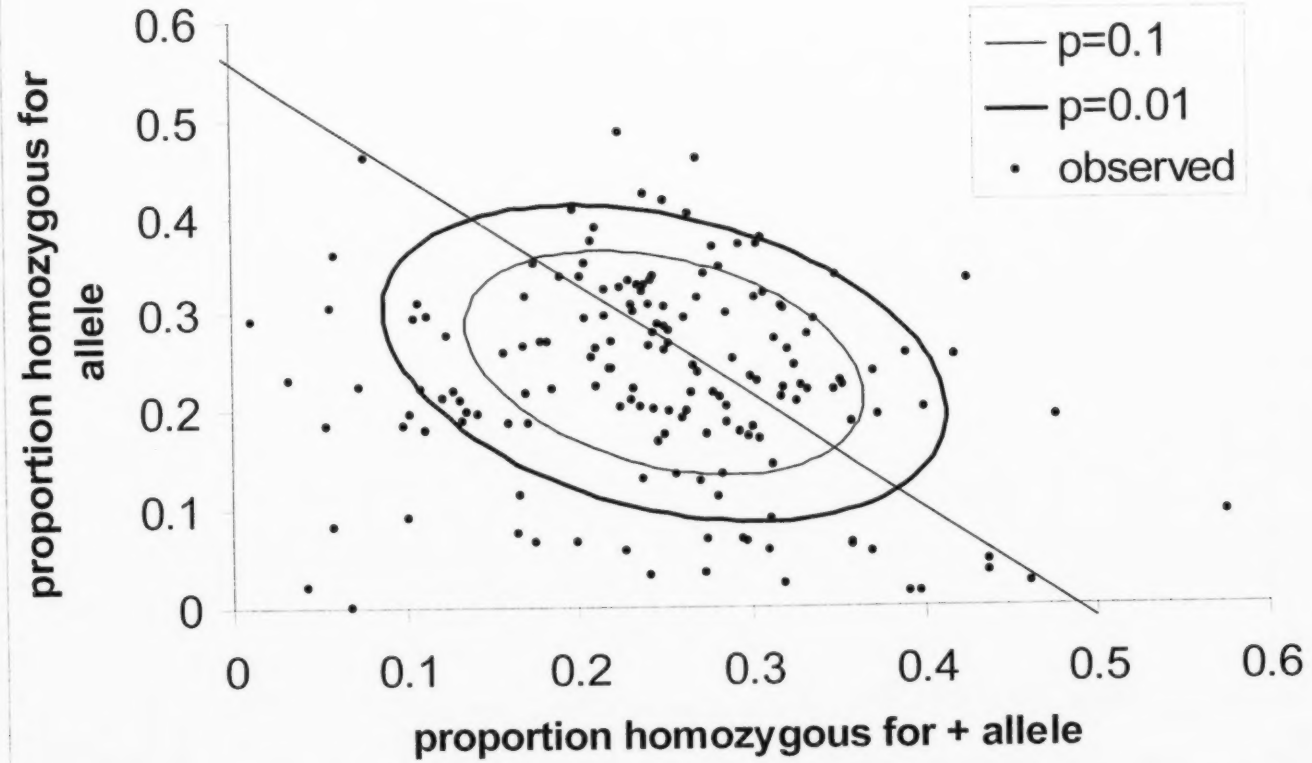


Figure 6: Correlation of Indole seed glucosinolate levels

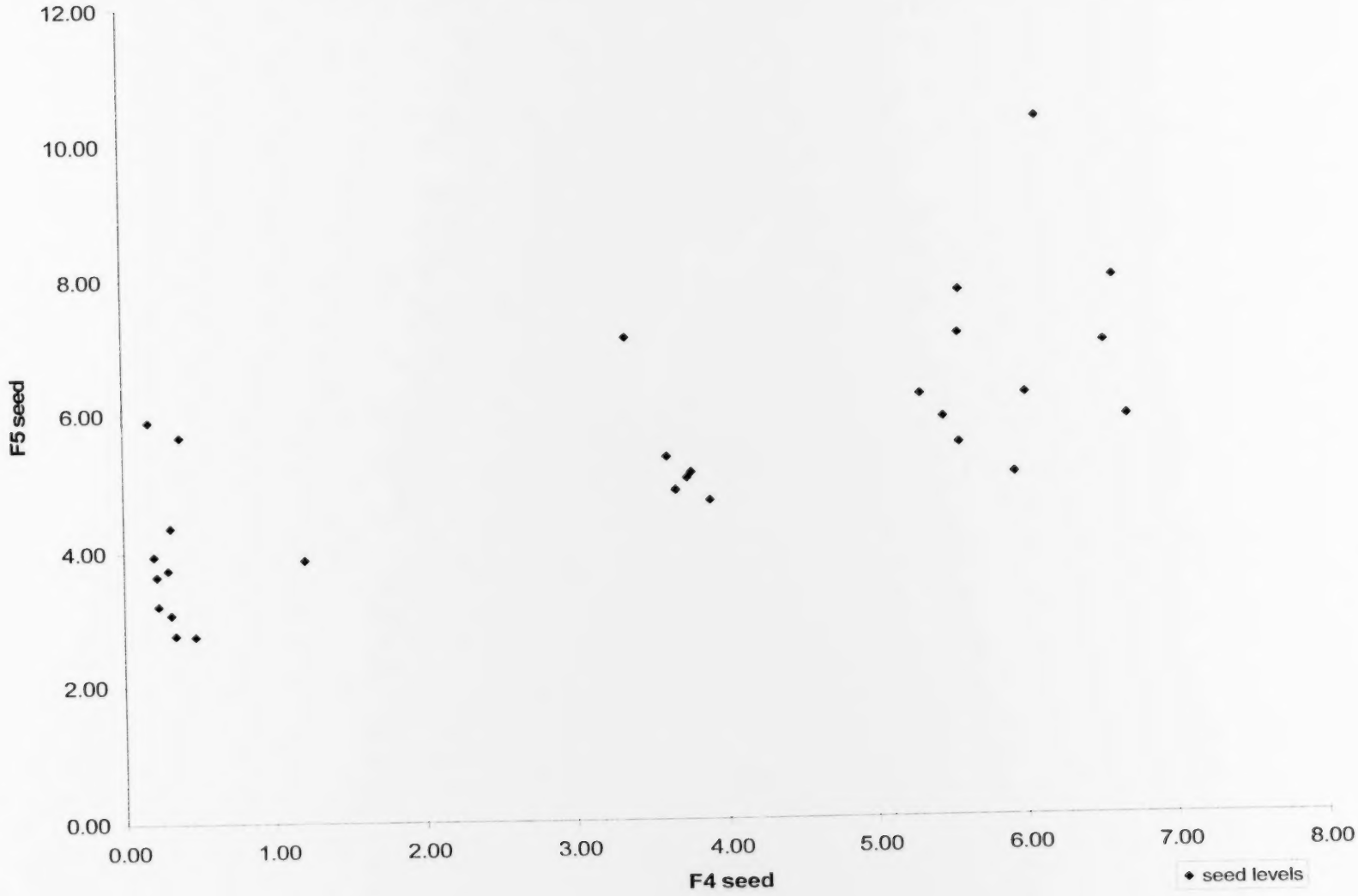
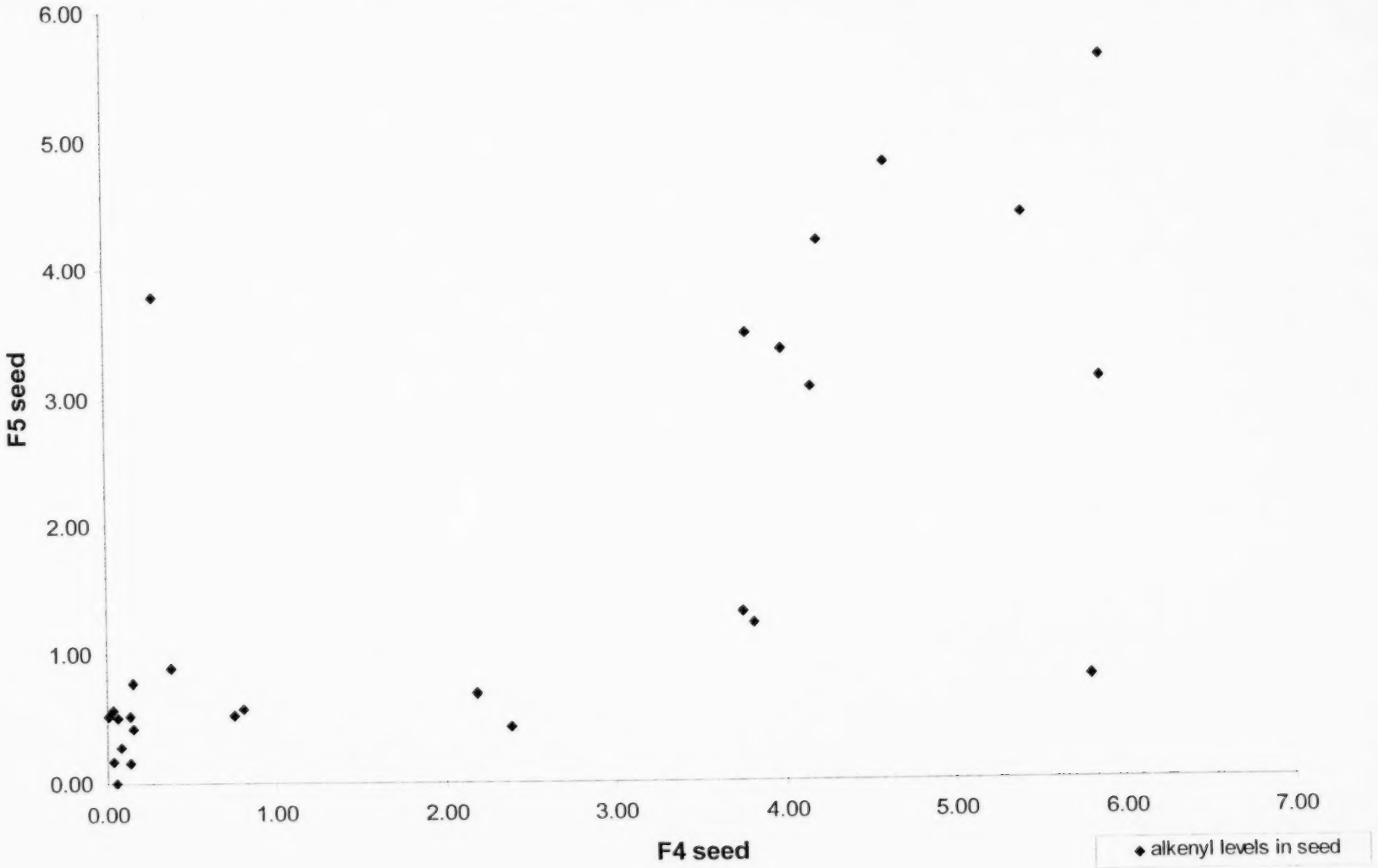


Figure 7: Correlation of Alkenyl glucosinolate levels in seed



40 entries for a 2 rep single row test. Randomize.

2001 Field Code	Rge	Row	Rep	Gen. Of Seed Planted	F3 Parent Plant	F2 Parent	10 seed bulk analysis on selfed F3 seed: GSL content(umoles/g seed) Bul	HOBut	Other	TotAlk	TotMI	TotInd	TOTGSL	% Fatty acid content C160	C180	C181	C182	C183	C200	C201	C221	SATD	F4 Seed Color	Agr. Rating 08/030/01	Bulk analysis on Seed Hvsted from the field 2001: Fatty acid content (%) C160	C180	C181	C182	C183	C200	C201	C221	SATD	Ratio1	Ratio2	GSL(umoles/g seed) Allyl	Butenyl	HOBut	Total Alkenyl	TOTMT	Total Indole	TOTGSL	% Oil Content
1742	46	1	1	F4	TO00-6321-8	5659-35	0.06	0.04	0.61	0.15	0.13	0.17	1.06	3.8	1.5	56.7	23.3	10.1	0.6	1.7	0.1	6.6	3.5	3	3.5	1.5	52.3	23.8	13.0	0.6	2.3	1.3	6.2	0.413	0.354	0.01	0.44	0.80	1.30	0.11	4.51	6.02	40.6
1742	47	1	2	F4	TO00-6321-8	5659-35	0.06	0.04	0.61	0.15	0.13	0.17	1.06	3.8	1.5	56.7	23.3	10.1	0.6	1.7	0.1	6.6	3.5	3	3.4	1.6	55.8	22.3	11.1	0.6	2.3	1.0	6.3	0.375	0.333	0.00	0.40	0.64	1.09	0.11	4.51	5.79	41.9
1743	46	2	1	F4	TO00-6331-6	5659-37	1.19	2.39	1.16	3.81	0.30	6.69	12.07	2.8	1.0	19.5	13.4	11.3	0.6	13.1	34.4	5.0	3.5	3												0.02	0.53	1.76	2.41	0.10	7.69	10.28	
1743	46	3	2	F4	TO00-6331-6	5659-37	1.19	2.39	1.16	3.81	0.30	6.69	12.07	2.8	1.0	19.5	13.4	11.3	0.6	13.1	34.4	5.0	3.5	3 plants-2	3.0	1.4	27.3	14.4	12.1	0.8	14.5	23.0	6.1	0.493	0.457	0.04	0.47	2.83	3.53	0.15	7.07	10.81	42.8
1744	46	3	1	F4	TO00-6331-7	5659-37	1.19	2.39	1.16	3.81	0.30	6.69	12.07	2.8	1.0	19.5	13.4	11.3	0.6	13.1	34.4	5.0	3.0	4	2.8	1.2	21.9	14.2	9.6	0.8	15.3	30.6	5.6	0.521	0.404	0.02	0.83	1.70	2.71	0.19	5.39	8.44	47.2
1744	46	3	1	F4	TO00-6331-7	5659-37	1.19	2.39	1.16	3.81	0.30	6.69	12.07	2.8	1.0	19.5	13.4	11.3	0.6	13.1	34.4	5.0	3.0	4	2.9	1.3	23.0	14.3	9.1	0.8	16.3	28.7	5.8	0.505	0.368	0.01	0.80	1.49	2.43	0.16	5.96	8.71	48.1
1744	46	4	2	F4	TO00-6331-7	5659-37	1.19	2.39	1.16	3.81	0.30	6.69	12.07	2.8	1.0	19.5	13.4	11.3	0.6	13.1	34.4	5.0	3.0	4	2.7	1.3	27.7	15.5	8.5	0.7	16.5	23.9	5.4	0.464	0.355	0.01	0.86	2.63	3.69	0.11	5.80	10.01	48.0
1745	46	4	1	F4	TO00-6332-7	5659-42	1.39	2.23	1.93	3.78	0.19	6.54	12.61	3.4	2.1	49.5	16.4	6.0	0.8	8.2	10.8	7.0		4	2.7	1.3	26.2	15.1	9.0	0.7	16.5	25.3	5.3	0.479	0.373	0.02	1.00	2.93	4.13	0.11	5.99	10.43	48.9
1745	47	5	2	F4	TO00-6332-7	5659-42	1.39	2.23	1.93	3.78	0.19	6.54	12.61	3.4	2.1	49.5	16.4	6.0	0.8	8.2	10.8	7.0	250+	4	2.7	1.3	25.6	15.7	7.9	0.8	17.2	25.4	5.6	0.480	0.334	0.00	0.68	1.35	2.17	0.22	8.92	11.43	48.7
1746	46	5	1	F4	TO00-6336-7	5659-125	0.78	3.12	0.08	4.16	0.10	6.10	10.57	2.7	1.0	23.1	15.8	8.8	0.6	13.7	30.7	4.8	2.0	4	2.9	1.3	24.7	15.4	8.1	0.8	17.0	26.7	5.4	0.487	0.344	0.03	0.61	1.31	2.06	0.24	8.00	10.59	49.7
1746	47	6	2	F4	TO00-6336-7	5659-125	0.78	3.12	0.08	4.16	0.10	6.10	10.57	2.7	1.0	23.1	15.8	8.8	0.6	13.7	30.7	4.8	2.0	3.5	2.7	1.3	24.7	15.4	8.1	0.8	17.0	26.7	5.4	0.487	0.344	0.03	0.61	1.31	2.06	0.24	8.00	10.59	43.9
1747	46	6	1	F4	TO00-6340-6	5659-183	0.27	0.40	0.40	0.75	0.04	6.60	8.44	2.5	1.2	28.7	12.5	7.9	0.6	12.8	30.4	4.7	3.5	3	3.1	1.6	32.6	17.1	10.2	0.8	11.8	19.6	6.2	0.456	0.374	0.01	0.27	0.88	1.24	0.43	8.47	10.29	46.8
1747	47	7	2	F4	TO00-6340-6	5659-183	0.27	0.40	0.40	0.75	0.04	6.60	8.44	2.5	1.2	28.7	12.5	7.9	0.6	12.8	30.4	4.7	3.5	3.5	2.9	1.4	25.9	15.1	9.2	0.8	11.5	29.5	5.9	0.485	0.379	0.01	0.46	1.12	1.73	0.29	8.18	10.38	45.7
1748	46	8	1	F4	TO00-6340-7	5659-183	0.27	0.40	0.40	0.75	0.04	6.60	8.44	2.5	1.2	28.7	12.5	7.9	0.6	12.8	30.4	4.7	1.0	2.5	2.6	1.2	21.9	15.4	7.6	0.8	13.5	33.1	5.5	0.513	0.330	0.01	0.15	0.55	0.75	0.27	5.80	6.95	45.7
1748	47	8	2	F4	TO00-6340-7	5659-183	0.27	0.40	0.40	0.75	0.04	6.60	8.44	2.5	1.2	28.7	12.5	7.9	0.6	12.8	30.4	4.7	1.0	3	2.7	1.3	24.4	15.5	8.2	0.8	15.8	27.8	5.5	0.493	0.346	0.01	0.39	1.43	1.93	0.21	7.96	10.52	46.6
1749	46	9	1	F4	TO00-6343-6	5659-30	1.00	4.56	1.06	5.88	0.23	3.75	11.03	2.5	0.9	28.5	16.5	9.6	0.5	10.9	27.3	4.3	3.0	3	2.6	1.1	22.4	13.6	10.2	0.6	13.1	32.6	5.1	0.516	0.429	0.03	1.21	3.43	5.06	0.26	5.77	11.30	43.5
1749	47	9	2	F4	TO00-6343-6	5659-30	1.00	4.56	1.06	5.88	0.23	3.75	11.03	2.5	0.9	28.5	16.5	9.6	0.5	10.9	27.3	4.3	3.0	3	2.7	1.0	18.8	13.6	11.3	0.6	11.9	36.1	5.0	0.570	0.452	0.06	1.71	3.96	6.40	0.40	6.26	13.35	43.1
1750	46	10	1	F4	TO00-6345-6	5659-67	0.31	4.89	0.70	5.41	0.15	3.67	9.97	2.8	1.0	25.8	15.7	11.8	0.4	12.9	26.0	4.7	4.5	3	3.1	1.6	32.6	17.1	10.2	0.8	11.8	19.6	6.2	0.456	0.374	0.01	0.27	0.88	1.24	0.43	8.47	10.29	43.9
1750	47	10	2	F4	TO00-6345-6	5659-67	0.31	4.89	0.70	5.41	0.15	3.67	9.97	2.8	1.0	25.8	15.7	11.8	0.4	12.9	26.0	4.7	4.5	4	2.9	1.4	25.9	15.1	9.2	0.8	11.5	29.5	5.9	0.485	0.379	0.01	0.46	1.12	1.73	0.29	8.18	10.38	46.8
1751	46	11	1	F4	TO00-6346-7	5659-166	0.75	10.31	0.10	11.71	0.02	3.35	15.21	2.1	0.7	17.8	13.0	10.5	0.4	8.8	41.9	4.0	4.0	4	3.0	1.4	35.8	16.8	10.1	0.7	11.8	17.8	5.7	0.429	0.374	0.01	0.33	0.55	0.93	0.10	3.17	4.25	43.6
1751	47	11	2	F4	TO00-6346-7	5659-166	0.75	10.31	0.10	11.71	0.02	3.35	15.21	2.1	0.7	17.8	13.0	10.5	0.4	8.8	41.9	4.0	4.0	4	3.0	1.4	32.8	16.8	10.1	0.7	11.8	17.8	5.7	0.429	0.374	0.01	0.33	0.55	0.93	0.10	3.17	4.25	45.4
1752	46	12	1	F4	TO00-6348-6	5659-221	1.41	4.31	0.34	5.86	0.41	3.78	10.44	3.4	1.6	63.9	19.0	7.8	0.7	1.6	0.1	6.3	3.5	3	3.0	1.5	37.1	16.1	9.5	0.7	11.8	17.3	5.9	0.408	0.372	0.01	0.18	0.33	0.54	0.06	2.99	3.71	43.7
1752	47	12	2	F4	TO00-6348-6	5659-221	1.41	4.31	0.34	5.86	0.41	3.78	10.44	3.4	1.6	63.9	19.0	7.8	0.7	1.6	0.1	6.3	3.5	4	3.0	1.5	39.9	16.3	9.3	0.7	11.4	15.3	6.0	0.390	0.363	0.00	0.22	0.42	0.66	0.06	3.03	3.90	44.3
1753	46	13	1	F4	TO00-6389-1	5659-92	0.01	0.03	0.00	0.04	0.00	0.69	0.73	3.0	1.3	35.0	14.7	8.0	0.7	14.3	19.6	5.6	3.5	3	3.6	1.8	56.3	20.2	10.8	0.6	3.0	1.7	6.7	0.355	0.349	0.00	2.34	2.83	5.38	0.38	6.41	12.43	42.2
1753	47	13	2	F4	TO00-6389-1	5659-92	0.01	0.03	0.00	0.04	0.00	0.69	0.73	3.0	1.3	35.0	14.7	8.0	0.7	14.3	19.6	5.6	3.5	3	3.5	1.9	57.0	20.2	10.6	0.6	2.8	1.6	6.6	0.350	0.344	0.03	2.33	3.25	5.77	0.38	6.32	12.75	42.2
1754	46	14	1	F4	TO00-6389-2	5659-92	0.01	0.03	0.00	0.04	0.00	0.69	0.73	3.0	1.3	35.0	14.7	8.0	0.7	14.3	19.6	5.6		2	2.3	0.9	22.0	14.5	9.7	0.6	12.0	34.4	4.6	0.524	0.400	0.03	1.24	4.02	5.81	0.15	6.10	12.55	45.2
1754	47	14	2	F4	TO00-6389-2	5659-92	0.01	0.03	0.00	0.04	0.00	0.69	0.73	3.0	1.3	35.0	14.7	8.0	0.7	14.3	19.6	5.6		2.5	2.3	0.9	23.5	14.0	9.2	0.6	12.1	33.9	4.5	0.496	0.398	0.02	0.92	4.07	5.55	0.30	5.80	11.90	45.6
1754	47	14	2	F4	TO00-6389-2	5659-92	0.01	0.03	0.00	0.04	0.00	0.69	0.73	3.0	1.3	35.0	14.7	8.0	0.7	14.3	19.6	5.6	2.5	3.5	2.9	1.4	32.3	17.1	9.9	0.7	12.4	20.3	5.7	0.455	0.367	0.01	0.35	0.67	1.08	0.08	2.59	4.21	44.7
1755	46	15	1	F4	TO00-6389-3	5659-92	0.01	0.03	0.00	0.04	0.00	0.69	0.73	3.0	1.3	35.0	14.7	8.0	0.7	14.3	19.6	5.6	2.5	2.5	2.6	1.1	22.4	13.6	10.2	0.6	13.1	32.6	5.1	0.516	0.429	0.03	1.21	3.43	5.06	0.26	5.77	11.30	43.5
1755	47	16	2	F4	TO00-6389-3	5659-92	0.01	0.03	0.00	0.04	0.00	0.69	0.73	3.0	1.3	35.0	14.7	8.0	0.7	14.3	19.6	5.6	2.5	few plants-3	3.1	1.5	37.8	18.3	9.3	0.7	10.9	15.6	5.9	0.423	0.337	0.01	0.73	1.24	2.07	0.13	2.99	5.29	
1756	46	16	1	F4	TO00-6389-4	5659-92	0.01	0.03	0.00	0.04	0.00	0.69	0.73	3.0	1.3	35.0	14.7	8.0	0.7	14.3	19.6	5.6	2.5	4	2.9	1.5	33.8	16.2	8.5	0.8	13.5	19.9	5.9	0.422	0.343	0.01	0.39	0.56	0.99	0.10	2.64	3.78	45.5
1756	47	17	2	F4	TO00-6389-4	5659-92	0.01	0.03	0.00	0.04	0.00	0.69	0.73	3.0	1.3	35.0	14.7	8.0	0.7	14.3	19.6	5.6	2.5	4.5	3.0	1.7	39.0	16.4	8.4	0.8	12.1	16.1	6.1	0.389	0.340	0.01	0.35	0.55	0.93	0.10	2.45	3.54	45.6
1757	46	18	1	F4	TO00-6390-1	5659-170	0.00	0.00	0.03	0.05	0.22	0.50	0.80	2.9	1.3	41.5	18.3	7.4	0.5	11.0	14.5	5.1		3	3.1	1.5	36.1	23.3	10.0	0.6	2.4	1.2	5.8	0.372	0.301	0.00	0.02	0.05	0.07	0.03	2.28	2.41	41.4
1757	47	18	2	F4	TO00-6390-1	5659-170	0.00	0.00	0.03	0.05	0.22	0.50	0.80	2.9	1.3	41.5	18.3	7.4	0.5	11.0	14.5	5.1		3	3.1	1.4	32.4	23.3	10.5	0.6	3.9	3.0	5.7	0.392	0.311	0.01	0.22	0.60	0.86	0.05	3.18	4.15	41.3
1758	46	19	1	F4	TO00-6393-1	5659-215	0.00	0.06	0.00	0.06	0.00	0.69	0.75	3.4	1.3	38.5	17.5	9.2	0.5	10.7	15.7	5.5	green	2	3.6	1.7	56.5	21.6	10.4	0.5	2.6	1.5	6.3	0.361	0.325	0.03	1.01	3.88	5.35	0.30	4.24	9.96	43.3
1758	47	19	2	F4	TO00-6393-1	5659-215	0.00	0.06	0.00	0.06	0.00	0.69	0.75	3.4	1.3	38.5	17.5	9.2	0.5	10.7	15.7	5.5	green	few plants-3	3.3	1.7	55.1	20.8	9.9	0.6	4.1	2.9	6.1	0.358	0.321	0.01	0.55	1.71	2.40	0.06	4.69	7.35	45.4
1759	46	20	1	F4	TO00-6393-2	5659-215	0.00	0.06	0.00	0.06	0.00	0.69	0.75	3.4	1.3	38.5	17.5	9.2	0.5	10.7	15.7	5.5	3	4	3.1	1.8	44.5	16.8	8.1	0.6	10.9	12.0	6.1	0.359	0.326	0.01	0.27	0.61	0.94	0.04	3.62	4.70	47.0
1759	47	20	2	F4	TO00-6393-2	5659-215	0.00	0.06	0.00	0.06	0.00	0.69	0.75	3.4	1.3	38.5	17.5	9.2	0.5	10.7	15.7	5.5	3	3.5	3.0	1.8	43.3	15.8	7.9	0.7	11.8	13.5	6.1	0.354	0.333	0.01	0.33	0.87	1.27	0.05	3.83	5.30	46.9
1760	46	21	1	F4	TO00-6393-4	5659-215	0.00	0.06	0.00	0.06	0.00	0.69	0.75	3.4	1.3	38.5	17.5	9.2	0.5	10.7	15.7	5.5		3	3.7	1.8	58.8	20.0	9.5	0.6	2.6	1.2	6.8	0.334	0.323	0.01	0.17	0.57	0.79	0.05	3.99	5.04	43.7
1760	47	21	2	F4	TO00-6393-4	5659-215	0.00	0.06	0.00	0.06	0.00	0.69	0.75	3.4	1.3	38.5	17.5	9.2	0.5	10.7	15.7	5.5		3	3.4	1.9	58.5	18.0	10.8	0.6	3.1	1.8	6.6	0.330	0.376	0.02	0.21	0.98	1.28	0.05	4.49	6.05	45.1
1760	47	21	2	F4	TO00-6393-4	5659-215	0.00	0.06	0.00	0.06	0.00	0.69	0.75	3.4	1.3	38.5	17.5	9.2	0.5	10.7	15.7	5.5		3	3.4	1.9	58.5	18.0	10.8	0.6	3.1	1.8	6.6	0.330	0.376	0.02	0.21	0.98	1.28	0.05	4.49	6.05	42.9
1761	46	23	1	F8	TO00P-6394-2	F7-0 GSL	0.01	0.00	0.26	0.01	0.18	0.62	1.10	2.5	1.2	20.9	10.8	6.5	0.8	8.7	43.7	6.0	3	few plants-2	2.9	1.0	19.0	14.3	9.1	0.7	11.8	37.2	5.5	0.552	0.388	0.00	0.00	0.00	0.00	0.05	0.99	1.06	42.9
1761	47	23	1	F8	TO00P-6394-2	F7-0 GSL	0.01	0.00	0.26	0.01	0.18	0.62	1.10	2.5	1.2	20.9	10.8	6.5	0.8	8.7	43.7	6.0	3	2	2.7	1.0	19.3	14.1	9.6	0.7	10.1	38.3	5.4	0.552	0.404	0.01	0.02	0.08	0.12	0.02	1.17	1.33	41.7
1762	46	24	1	F8	TO00P-6394-3	F7-0 GSL	0.01	0.00	0.26	0.01	0.18	0.62	1.10	2.5	1.2	20.9	10.8	6.5	0.8	8.7	43.7	6.0	2.5	few plants-2	2.6	0.9	17.9	14.3	10.0	0.6	10.3	39.0	5.2	0.576	0.411	0.01	0.00	0.02	0.03	0.00	1.13	1.18	42.1
1762	47	23	2	F8	TO00P-6394-3	F7-0 GSL	0.01	0.00	0.26	0.01	0.18	0.62	1.10	2.5	1.2	20.9	10.8	6.5	0.8	8.7	43.7	6.0	2.5	1.5	2.4	0.8	16.9	14.1	10.5	0.6	9.4	40.7	5.0	0.592	0.427	0.00	0.01	0.05	0.06	0.05	1.86	1.99	41.6
1763	46	25	1	F8	TO00P-6394-4	F7-0 GSL	0.01	0.00	0.26	0.01	0.18	0.62	1.10	2.5	1.2	20.9	10.8	6.5	0.8	8.7	43.7	6.0	2.5	2	2.6	1.0	19.4	14.3	9.6	0.7	11.4	36.9	5.4	0.552	0.402	0.00	0.00	0.00	0.05	0.16	1.64	1.87	41.8
1763	47	24	2	F8	TO00P-6394-4	F7-0 GSL	0.01	0.00	0.26	0.01	0.18	0.62	1.10	2.5	1.2	20.9	10.8	6.5	0.8	8.7	43.7	6.0	2.5	1.5	2.6	1.1	20.9	14.7	9.8	0.7	11.2	34.8	5.4	0.540	0.401	0.05	0.00	0.00	0.05	0.16	1.64	1.87	39.6
1764	46	26	1	S5	TO00P-6395-1	S4-YN95-663	0.06	0.13	0.15	0.20	0.17	5.37	6.12	4.0	1.7	60.2	22.2	8.2	0.6	1.3	0.0	6.9	gr	3	3.5	2.0	60.7	21.2	8.1	0.6	1.6	0.7	6.7	0.326	0.276	0.01	2.80	3.63	6.74	0.31	8.97	16.42	46.8
1764	47	25	2	S5	TO00P-6395-1	S4-YN95-663	0.06	0.13	0.15	0.20	0.17	5.37	6.12	4.0	1.7	60.2	22.2	8.2	0.6	1.3	0.0	6.9	gr	3.5	3.6	2.0	62.3	20.4	7.7	0.6	1.5	0.2	6.8	0.311	0.274	0.02	2.19	2.99	5.45	0.26	8.17	14.32	47.6
1765	46	27	1	S5	TO00P-6395-2	S4-YN95-663	0.06	0.13	0.15	0.20	0.17	5.37	6.12	4.0	1.7	60.2	22.2	8.2	0.6	1.3	0.0	6.9	gr	3	3.5	2.0	61.8	20.7	7.9	0.6	1.5	0.4	6.7	0.317	0.276	0.12	2.89	3.83	7.15	0.36	8.36	16.33	47.2
1765	47	26	2	S5	TO00P-6395-2	S4-YN95-663	0.06	0.13	0.15	0.20	0.17	5.37	6.12	4.0	1.7	60.2	22.2	8.2	0.6	1.3	0.0	6.9	gr	3	3.5	2.1	63.1	19.7	7.7	0.6	1.5	0.2	6.8	0.302	0.280	0.03	2.51	3.27	6.07	0.30	8.44	15.30	47.6
1766	46	28	1	S5	TO00P-6395-3	S4-YN95-663	0.06	0.13	0.15	0.20	0.17	5.37	6.12	4.0	1.7	60.2	22.2	8.2	0.6	1.3	0.0	6.9	2	4.5	3.6	1.9	60.8	21.4	8.1	0.6	1.5	0.3	6.8	0.327	0.275	0.02	2.37	3.15	5.82	0.36	8.63	15.26	46.5
1765	47	26	2	S5	TO00P-6395-3	S4-YN95-663	0.06	0.13	0.15	0.20	0.17	5.37	6.12	4.0	1.7	60.2	22.2	8.2	0.6	1.3	0.0	6.9	2	3.5	3.6	2.0	62.2	20.4	7.7	0.6	1.6	0.3	6.8	0.311	0.274	0.00	2.46	3.30	6.06	0.31	8.87	15.76	47.2
1766	46	28	1	S5	TO00P-6395-3	S4-YN95-663	0.06	0.13	0.15	0.20	0.17	5.37	6.12	4.0	1.7	60.2	22.2	8.2	0.6	1.3	0.0	6.9	2	4.5	3.7	2.0	62.1	20.8	7.6	0.6	1.5	0.2	6.9	0.313	0.267	0.02	2.24	2.72	5.20	0.25	8.39	14.30	46.4
1766	47	27	2	S5	TO00P-6395-3	S4-YN95-663	0.06	0.13	0.15	0.20	0.17	5.37	6.12	4.0	1.7	60.2	22.2	8.2	0.6	1.3	0.0	6.9	2	3.5	3.7	2.1	62.5	20.5	7.5	0.6	1.4	0.2	6.9	0.309	0.269	0.02	2.13	2.61	4.96	0.25	8.44	14.12	46.9
1767	46	29	1	S5	TO00P-6395-4	S4-YN95-663	0.06	0.13	0.15	0.20	0.17	5.37	6.12	4.0	1.7	60.2	22.2	8.2	0.6	1.3	0.0	6.9	2	3.5	3.7	2.1	62.5	20.5	7.5	0.6	1.4	0.2	6.9	0.309	0.269	0.02	2.13	2.61	4.96	0.25	8.44	14.12	47.7
1767	47	28	2	S5	TO00P-6395-4	S4-YN95-663	0.06	0.13	0.15	0.20	0.17	5.37	6.12	4.0	1.7	60.2	22.2	8.2	0.6	1.3	0.0	6.9	2	5	3.4	1.8	61.7	17.3	8.8	0.6	3.1	1.6	6.5	0.297	0.337	0.02	1.54	2.44	4.13	0.14	6.84	11.28	48.7
1768	46	7	1	Excel	TO00P-6396-1	AC Excel(93-cert sd)																	5	4	3.4	1.8	63.9	17.3	8.9	0.6	1.9	0.6	6.4	0.291	0.339	0.03	2.11	3.57	5.94	0.25	6.74	13.21	45.3
1768	47	29	2	Excel	TO00P-6396-1	AC Excel(93-cert sd)																	5	5	3.5	1.7	62.7	18.6	9.1	0.6	1.8	0.4	6.4	0.307	0.329	0.02	1.40	2.29	4.26	0.14	6.70	11.37	46.7
1769	46	22	1	Excel	TO00P-6396-2	AC Excel(93-cert sd)																	5	5	3.4	1.8	63.6	17.7	9.0	0.6	1.9	0.5	6.4	0.295	0.336	0.01	1.48	2.26	4.36	0.14	7.38	12.15	46.7
1769	47	15	2	Excel	TO00P-6396-2	AC Excel(93-cert sd)																		3	3.0	1.6	37.9	17.4	10.1	0.6	10.6	16.1	5.8	0.421	0.367	0.00	0.22	0.39	0.65

primer name	Clone_Name	locus	linkage_group	allele_size_101	allele_size_109	allele_size_102
sS1940	sS1940	a	N8	165	149	
sS1921	sS1921		N8		187	226
sR1868	sR1868	a				
sR1868	sR1868	b				
sNRF19	sNRF19	a	N8		120	85
sN1087	sN1087	b	N17	467	453	
sN1087	sN1087	a	N8		461	472
sN1087	sN1087	cNP	N3		454	
sN0809	sN0809		N8		314	379
sN1834	sN1834	cNP	N11			213
sN1834	sN1834	bNP	N2			244
sN1834	sN1834	cNP	N11		214	
sN1834	sN1834	d	N19		177	179
sN1834	sN1834	fNM	N13			229
sN1834	sN1834	eNM	N19			252
sN1834	sN1834	aNP	N19			
sS1725	sS1725	bNM	N1	110		
sS1725	sS1725	a	N11	149	162	
sN1838	sN1838	a	N11		231	229
sN1838	sN1838	bNM	N1			202
sNRF94	sNRF94		N11	285	310	
sNRC24	sNRC24					
sORD34	sORD34		N14	204	219	
sORD34	sORD34					
sORD34	sORD34					
sORG90	sORG90		N14		216	256
sN0412	sN0412	a	N14		247	241
sN0412	sN0412	b	N4		241	222
sNRB93	sNRB93	bNP	N17			116
sNRB93	sNRB93	aNP	N6		141	
sNRB93	sNRB93	b	N17	123	117	
sORE87	sORE87	bNP	N6		113	
sORE87	sORE87	b	N6		113	136
sORE87	sORE87	a	N17		120	113
sN3603	sN3603	a	N6	325	340	
sN3603	sN3603	a	N6		340	321

sNRG62	sNRG62	bNM	N11	140		
sNRG62	sNRG62	a	N14	287	285	
sS1852	sS1852		N14		185	193
sS2066	sS2066	a	N10		241	244
sS2066	sS2066	b	N19		221	219
sNRG88	sNRG88					
sNRG88	sNRG88	a	N10		166	158
sNRG88	sNRG88	aNP	N10	166		
sS2090	sS2090		N10		100	137
sS2090	sS2090		N10	107	99	
sNRF87	sNRF87		N10	131	124	
sN1113	sN1113	aNM	#2 Nulls			
sORH13	sORH13		N15		274	256/203
sS2165	sS2165	a	N6		214	179
sS2165	sS2165	b	N5		175	160
sNRB46	sNRB46		N6		150	127
sN2837	sN2837	a	N6		159	165
sN2837	sN2837	a	N6	154	160	
sN2426	sN2426	NP	N6		289	
sORG41	sORG41	aNP	N6		106	
sN1914J	sN1914J	a	N8		324	357
sN1914J	sN1914J	b	N6		301,281	250,257
sNRF36	sNRF36	aNP	N14		127	124
sORE67	sORE67	a	N14		131	129
sORB74	sORB74	a	N14		128	
sNRA95	sNRA95	aNP	N7		236	
sNRA95	sNRA95	cNM	N9			169/173
sNRA95	sNRA95	bNM				255
sS1907	sS1907	bNM	N4	149		
sS1907	sS1907	cNM	N5	124		
sS1907	sS1907	a	N16	94	101	
sNRD57	sNRD57					
sNRD57	sNRD57	a	N2	161	166	
sNRD57	sNRD57					
sORE27	sORE27	bNM	N12			224
sORE27	sORE27	aNP	N2		219	
sORB56	sORB56	bNM	N12			231

sORB56	sORB56	aNP	N12		248	
sNRE85	sNRE85	aNP	N2		202	
sNRE85	sNRE85	bNM	N2			181
sORE30	sORE30	a	N19		127	119
sN2025	sN2025		N4	128	136	
sN2025	sN2025		N4		136	125
sR2103	sR2103	b	N12		275	301
sR2103	sR2103	a	N2		289	301
sORB10	sORB10	a	N17		271	273
sORB10	sORB10	b	N18		60	66
sNRE74	sNRE74	a	N12	154	165	
sR2028	sR2028	bNP	N2		256	
sR2028	sR2028	cNM	N12			262
sR2028	sR2028	dNM	N2			250
sR2028	sR2028	aNP	N12		264	
sN0583	sN0583	aNM	N14		361	
sN0583	sN0583	bNP	N12		371	
sN0583	sN0583	c	N2		369	367
sN1982	sN1982	b	N2		145	147
sN1982	sN1982	aNP	N19		154	
sN1982	sN1982	c	N10		139	141
sN1982	sN1982	dNM	N2			137
sORC76	sORC76	aNP	N12		126	
sORC76	sORC76	bNM	N19			120
sORC76	sORC76	cNM	N17			116
sORC76	sORC76	dNP	N13		106	
sN1925	sN1925	dNM	N2			338
sN1925	sN1925	aNP	N12		365	
sN1925	sN1925	bNP	N2		341	
sN1925	sN1925	cNM	N12			356
sORA82	sORA82	bNP	N2		83	
sORA82	sORA82	a	N12		104	108
sN2320	sN2320	c	N17	121	127	
sN2320	sN2320	aNP	N2		147	
sN2320	sN2320	a	N2	154	147	
sN2320	sN2320	b	N12	137	132	
sN2168	sN2168	aNP	N12		248	

sN2168	sN2168	bNM	N12			245
sN1046F	sN1046F	a	N13		201	213
sN1046F	sN1046F	a	N13	237	202	
sS2352	sS2352	a	N9		170	227
sN3609F	sN3609F					187
sN3609F	sN3609F	aNM	N12		193	171
sN3886I	sN3886I	a	N12		165	169
sN3886I	sN3886I	b	N10		170	
sN3886I	sN3886I	cNP	N19		338	334
sN3916	sN3916	a	N2			
sN3916	sN3916				327	334
sN3916	sN3916	b	N12		393	
sN8836	sN8836	aNP	N12		288/278/274	272
sN3734	sN3734	a	N11		272	270
sN3672R	sN3672R		N2			
sN3672R	sN3672R		N2		181	175
sNRC03	sNRC03	a	N15		334	327
sN1963	sN1963	a	N5			
sN1963	sN1963				249	
sN8360	sN8360	cNP	N5			300
sN8360	sN8360	dNM	N9		270	
sN8360	sN8360	aNP	N9			276
sN8360	sN8360	eNM	N10		268	
sN8360	sN8360	bNP	N4			148
sNRC89	sNRC89	cNM	N14		169	
sNRC89	sNRC89	aNP	N12		141	138
sNRC89	sNRC89	d	N18		165	155
sNRC89	sNRC89	b	N9			
sNRD50	sNRD50				126	
sNRD50	sNRD50		N7	91		401
sN3620	sN3620	bNM	#2 Nulls		396	
sN3620	sN3620	aNP	N7		118	89
sS1864	sS1864		N19	102	117	
sS1864	sS1864				362	349
sN1839	sN1839	b	N9		391	376
sN1839	sN1839	a	N19			
sNRG42	sNRG42		N19	206	205	

sNRG42	sNRG42					
sNRG42	sNRG42		N19	206	253	
sN0653	sN0653		N19	196	199	
sN2016	sN2016		N19	265	271	
sN2016	sN2016		N19		270	264
sORC88	sORC88		N19		131	133
sNRH22	sNRH22	a	N11		233	165/148
sNRF22	sNRF22	b	N9		137	135
sNRF22	sNRF22	a	N18		156	144
sR2181	sR2181		N7	147	139	
sNRH21	sNRH21	aNP	N5		179	
sNRH21	sNRH21					
sNRH21	sNRH21	b	N17	170	172	
sNRH21	sNRH21					
sNRH21	sNRH21	a	N5	123	179	
sNRH21	sNRH21	b	N17		172	171
sR2064	sR2064	a	N15		104	102
sR2064	sR2064					
sS2362	sS2362	cNM	N11			110
sS2362	sS2362	bNP	N19		133	
sS2362	sS2362	a	N13		139	121
sN0855	sN0855	a	N14	374	368	
sN0855	sN0855	a	N14		368	360
sN0990	sN0990	aNP	N14		181	
sN0990	sN0990	bNP	N1		161	
sORG31	sORG31		N14		105	123
sN10326	sN10326	a	N14		185	172
sN0584	sN0584		N14		363	361
sN4638	sN4638		N1		491	494
sN2305	sN2305	a	N1		342	351
sN2305	sN2305	b	N11		347	307
sN7974	sN7974	bNM	N1			228
sN7974	sN7974	dNM				234
sN7974	sN7974	aNM	N11	235		
sN7974	sN7974	cNM	N1	250		
sN3523r	sN3523R	b	N1		212	217
sN3523r	sN3523R	a	N11	225	219	

sN3523r	sN3523R	b	N1	205	212	
sN3523r	sN3523R	a	N11		220	228
sN8607	sN8607	a	N13	471	454	
sN8607	sN8607	bNM	N1	140		
sNRD03	sNRD03					
sNRD03	sNRD03		N5	93	99	
sN0539	sN0539	aNP	N6	210	202	
sN0539	sN0539	a	N6		205	
sN1944	sN1944	a			291	285
sN1944	sN1944	bNM	N6		321	
sR2223	sR2223	bNP	N3		305	
sR2223	sR2223					
sR2223	sR2223	a	N13	315	309	
sR2223	sR2223					
sN1937	sN1937	aNP	N12		346	
sN3274I	sN3274I	dNP	N5		232	
sN3274I	sN3274I	cNP	N11		247	
sN3274I	sN3274I	eNP	N12		209	
sN3274I	sN3274I					223
sN3274I	sN3274I	gNM	N18			214
sN3274I	sN3274I	hNM	N17			211
sN3274I	sN3274I	iNM	N12			178
sN3274I	sN3274I	jNM	N14			
sN3274I	sN3274I	aNP	N5		298	
sN3274I	sN3274I	bNP	N12		292	
sNRE30	sNRE30	cNP	N2		175	
sNRE30	sNRE30	bNM	N2			191
sNRE30	sNRE30	aNP	N12		204	
sNRB38	sNRB38		N15		124	119
sNRC10	sNRC10		N19		203	170
sNRB47	sNRB47	a	N6		204	192
sNRB47	sNRB47	bNP	N5		157	
sNRB47	sNRB47	cNP	N13		79	
sNRB47	sNRB47					
sNRG54	sNRG54	aNP	N13		111	
sNRG54	sNRG54	bNP			98	
sNRC57	sNRC57	a	N9		189	200

sS1718	sS1718	a	N10		131	
sORH62	sORH62	NP	N10		280	
sNRH84	sNRH84	NP	N14		118	
sORD65	sORD65	a	N17		319	334
sORE95	sORE95	NM	N14		107	
sN3277	sN3277	bNP	N14		355	
sN3277	sN3277	aNP	N13		375	
sN0105	sN0105	bNM	N13			198
sN0105	sN0105	a	N11		157	214
sS2141	sS2141	dNM	#2 Nulls		446	
sS2141	sS2141	aNP	N16		129	
sS2141	sS2141	bNM	N7	137		
sS2141	sS2141	cNP	N7		414	
sORG49	sORG49	a	N9	148	139	
sORG49	sORG49	b	N9	152	139	
sN0319	sN0319	a	N15		289	249
sN0319	sN0319	bNP	N12		218	
sN0319	sN0319	cNM	N12			269
sN0319	sN0319	dNM	N3			202
sORE79	sORE79	bNM				114
sORE79	sORE79	aNP	N14		116	
sN0740	sN0740		N14		235/218	223/218
sN2315	sN2315	bNP	N4		145	
sN2315	sN2315	a	N3		158	156
sNRG06	sNRG06	a	N7	237	232	
sNRG06	sNRG06	b		225	237	
sNRG06	sNRG06	cNM	N2	192		
sN0786	sN0786	b	N3		265	264
sN0786	sN0786	a	N4		443	472
sN1915	sN1915	b	N3		334	329
sN1915	sN1915					
sN1915	sN1915	a	N13		332	341
sN0857	sN0857	dNM				287
sN0857	sN0857	a	N3		311/295	305/239
sN0857	sN0857	b	N12		264	271
sN0857	sN0857	cNM	N2			374
sN0810	sN0810	a	N3		315	313

sN0810	sN0810					
sNRA84	sNRA84		N11	221	219	
sS2082	sS2082	bNM	N9	317		
sS2082	sS2082	aNP	N11		277	
sS2082	sS2082	c	N9		291	312
sS2082	sS2082	d	N18		291	296
sN0238	sN0238	aNM	N18		77	
sN2189	sN2189	b	N6		354	366
sN2189	sN2189					
sN2189	sN2189	aNP	N18		352	
sN0248I	sN0248I	a	N11		144	151
sN7644	sN7644					
sN7644	sN7644	a	N14		366	364
sN8591	sN8591	a	N3		335	230
sN3521f	sN3521F					
sN3521f	sN3521F					
sN3521f	sN3521F	b	N15		147	162
sN3521f	sN3521F	a	N5		162	122
sN3533I	sN3533I	c	N19	294	288	
sN3533I	sN3533I					
sN3533I	sN3533I	bNM	N11			308
sN3533I	sN3533I	aNP	N12		362	
sN3533I	sN3533I	cNP	N19		288	
sN3533I	sN3533I	bNM	N11	308		
sN3533I	sN3533I	aNP	N12		362	
sN8723	sN8723	aNM	N18			252
sN8723	sN8723	bNM	N11	253		